

1 **Clonal thermal preferences affect the strength of the temperature-size rule**

2 **Running title:** Thermal preference affects temperature-size rule

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4 Anna Stuczyńska^{1,2}, Mateusz Sobczyk¹, Edyta Fiałkowska¹, Wioleta Kocerba-Soroka¹,
5 Agnieszka Pajdak-Stós¹, Joanna Starzycka¹, Aleksandra Walczyńska¹

6

7

8 ¹ Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Krakow,
9 Poland

10 ² Centrum for Biomarker Research (CeBioR), University of Huddersfield, Queensgate,
11 Huddersfield HD1 3DH, UK

12

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21 **Abstract**

22 Genetically similar organisms act as a powerful study system for the subtle differences in various
23 aspects of life histories. The issue of trade-offs among traits is of special interest. We used six
24 parthenogenetic rotifer clones previously exposed to different thermal laboratory conditions.
25 Interclonal differences in female body size were examined in common garden conditions. We
26 estimated the population growth rate and strength of the size-to-temperature response across four
27 thermal regimes. We tested hypotheses on the existence of the relationships between (i) thermal
28 acclimation and species body size, (ii) thermal specialization and fitness and (iii) thermal
29 specialization and strength of the temperature-size rule. Positive verification of (i) would make it
30 justifiable to refer the other investigated traits to thermal preference and, further, to thermal
31 specialization. Addressing the issues (ii) and (iii) is our pioneering contribution to the question on
32 the strength of size-to-temperature response as differing across life strategies. We hypothesized
33 that this plastic response may be affected by the level of thermal specialization and that this
34 pattern may be traded off with the temperature-dependent potential for population growth rate.
35 Additionally, we investigated the differences in reproductive strategy (number of eggs laid by a
36 female and female lifetime duration) in one temperature assumed optimal, which acts as an
37 important supplement to the general clonal life strategy. We confirmed that the thermal
38 acclimation of a clone is related to body size, with clones acclimated to higher temperatures
39 being smaller. We also found that warm-acclimated clones have a narrower thermal range (= are
40 more specialized), and that the temperature-size rule is stronger in rotifers acclimated to
41 intermediate thermal conditions than in specialists. Our results contribute into the issue of trade-
42 offs between generalist and specialist strategies, in the context of plastic body size response to
43 different temperatures.

44 **Keywords:** body size, generalists, phenotypic plasticity, rotifers, specialists, thermal
45 specialization

46

47 **Introduction**

48 Optimal allocation theory states that living organisms optimize their strategies of the allocation of
49 limited resources among the functions associated with maintenance, repair and reproduction. Any
50 given set of abiotic and biotic conditions provides a unique, optimal strategy, and trade-offs
51 among life history traits are at the base of such strategies (Cody 1966; Kozłowski 1992;
52 Kozłowski 2006; Roff 1992; Stearns 1992). The pattern of limited resources causing the traits to
53 trade is complex and troublesome with regard to straightforward inferences and conclusions
54 (Acerenza 2016; Lailvaux and Husak 2017; Roff and Fairbairn 2007). Going a step further,
55 DeWitt (2016) stressed the importance of including not only trait means but also the shape (i.e.,
56 skew) of phenotypic plasticity in studies on life-history optimization in ecological contexts.
57 According to the author, the strength of phenotype distribution across environments should be
58 optimized by means of natural selection. Phenotypic plasticity is a non-random variance in a
59 given trait that does not involve differences in genotype, an idea that has been well established
60 since Levins (1968). However, this idea is still not well integrated with life-history theory
61 (DeWitt 2016). In this study, we investigated how the strength of the phenotypic size-to-
62 temperature response varies in six parthenogenetic rotifer clones previously exposed to different
63 temperatures in the laboratory.

64 The phenotypic achievement of smaller body size with increasing temperature in ectotherms is a
65 widely observable but not fully understood phenomenon called the temperature-size rule (TSR,

66 Atkinson 1994). Revealing the adaptive significance of this pattern is one of the largest
67 challenges facing evolutionary biology (Berrigan and Charnov 1994), especially given that it
68 concerns one of the most important organismal traits, body size (Hildrew et al. 2007; Kozłowski
69 2006; Stearns 1992), and one of the most evolutionarily influential environmental variables,
70 temperature (Johnston and Bennett 2008). Direct confirmation of the adaptive significance of the
71 TSR was provided by Walczyńska et al. (2015a) for *Lecane inermis* rotifer. In the first step of our
72 study focusing on the same species, our goal was to confirm whether thermal acclimation is
73 linked with thermal preference through body size at the interclonal level, namely, whether the
74 clones prefer the temperature they were acclimated to. Given that the TSR is the adaptive body
75 size adjustment to given thermal conditions, clones acclimated to lower/higher temperatures
76 should prefer the respective thermal range, and further, those acclimated to lower/higher
77 temperature should display larger/smaller body sizes, respectively.

78 The next step of the study was to link thermal preference with thermal specialization through
79 fitness. We predicted that clones preferring low or high temperature would specialize to the
80 respective thermal range and would perform better (= have higher fitness) at those respective
81 temperatures than other clones. This prediction is based on the optimal allocation theory, which
82 assumes the existence of resource limitations that force trade-offs and consequently diversify the
83 life strategies of organisms. Some historical predictions were made regarding the level of
84 ecological specialization and performance. The most classical one was proposed by Levins
85 (1968). He assumed a distinction between generalists, characterized by relatively high and flat
86 performance across environments, and specialists, which perform better in one type of
87 environment than in others. This idea was challenged multiple times, and the thermal biology
88 field displays both empirical evidence in favor of (Foray et al. 2011; Griffith and Sultan 2006;

89 Verberk et al. 2010; Walczyńska and Serra 2014) and against predictions (Berger et al. 2014;
90 Knies et al. 2009; Sheth and Angert 2014). However, the most intuitive argument in favor of the
91 existence of such a trade-off came from work on the temperature dependence of proteins (Fields
92 2001), which showed that enzymes can be either molecularly stable (a “specialist”) or structurally
93 flexible (a “generalist”) depending on the physical role they play, but not both. Therefore,
94 enzymes adapted to work at one temperature will be unable to function at suboptimal
95 temperatures unless their structure is modified (Fields 2001).

96 In the final step, we switched from the single phenotype to the variable phenotypes perspective,
97 namely, phenotypic plasticity. Divergent natural selection resulting from environmental variation
98 creates functional trade-offs between environments; organisms adapted to one environment
99 present reduced performance in alternative environments (DeWitt and Langerhans 2004).

100 Moreover, theoretical modeling predicts that the strategy of intermediate phenotypes, the
101 generalists, is to reduce variance in performance across environments (DeWitt and Langerhans
102 2004). We use this theoretical background for the first time in the context of the strength of
103 plastic size response to temperature. We test this novel suggestion on six clones of *Lecane*
104 *inermis* rotifer species.

105 Under these theoretical assumptions, we aimed to match the general interclonal potential for
106 phenotypic plasticity in size response to temperature with performance measured in the most
107 universal evolutionary currency, fitness. To date, there is no information in the literature on the
108 possible trading off between the size-to-temperature response and general thermal preferences,
109 except for some suggestions presented by Walczyńska and Serra (2014). The authors suggested
110 that the differences in the strength of the TSR response found in three *Brachionus* species
111 belonging to the *Brachionus plicatilis* cryptic species complex might be attributed to the level of

112 species thermal specialization, with generalists showing more flexible plastic body size
113 adjustment across temperature than specialists (Walczyńska and Serra 2014).

114 Clones, which are highly genetically similar organisms, provide a promising system for
115 understanding the subtle details behind the observed eco-evolutionary processes. The interclonal
116 perspective in studies on the temperature effect was previously successfully applied to
117 reproductive strategy in the *Lecane inermis* rotifer studied here (Fiałkowska et al. 2011) and to
118 size at maturity and asymptotic size (Hoefnagel et al. 2018) and vital rates (Bruijning et al 2018)
119 in *Daphnia magna*. In this study, we explored the unique opportunity of possessing in the
120 laboratory six clones of *Lecane inermis* Bryce (Rotifera) that were previously exposed to
121 different thermal regimes. We assumed that they exemplified different thermal preferences
122 gained through adaptive mechanisms. Our reasoning involved treating the clone-specific body
123 size information as a link between thermal acclimation (warm-, intermediate temperature- or
124 cold-acclimated clones) and thermal preference, based on the theoretical predictions as described
125 above. If our predictions are correct, this linkage would justify that we refer our results to the
126 clonal thermal preferences. We estimated the thermal sensitivity of both the population growth
127 rate r and the size-to-temperature response across the same four thermal regimes. Additionally,
128 we searched for possible differences in the clone-specific reproductive strategy of females
129 (number of eggs laid and duration of life) at the temperature assumed optimal. Such differences
130 would facilitate the interpretation of the results on the general performance and the abilities of
131 plastic response to environmental changes.

132 Based on the theoretical predictions on the performance of generalists vs. specialists in different
133 environments (DeWitt and Langerhans, 2004; Levins, 1968) we hypothesized the following
134 patterns in phenotypic size-to temperature response (Atkinson,1994):

135 H1: body size of clones differs according to their thermal acclimation; cold-acclimated clones are
136 the largest, followed by intermediate clones, and the smallest are warm-acclimated clones;
137 H2: cold- and warm-acclimated clones are more specialized (= have higher fitness) to lower or
138 higher temperatures, respectively, than clones acclimated to intermediate temperatures (=
139 generalists);
140 H3: phenotypic size response to temperature (TSR) is stronger in clones acclimated to
141 intermediate temperatures than in more specialized cold-acclimated and warm-acclimated clones.

142

143 **Material and Methods**

144 **Clones isolation and maintenance.** *Lecane inermis* is a bacterivorous, monogonont rotifer
145 species occurring in psammolittoral fresh and brackish water bodies that is relatively common
146 worldwide (Bielańska-Grajner et al. 2015). It is a frequent inhabitant of wastewater treatment
147 plants (Klimowicz 1989), from which all the investigated clones were isolated. Its lifecycle
148 consists of the sexual and asexual phases (Miller 1931), although in most cases, the sexual phase
149 disappears in the populations from the wastewater treatment plants (Pajdak-Stós et al. 2014). The
150 generation time estimated for one clone is approximately 2 days between 15 °C and 25 °C
151 (Walczyńska et al. 2015b), while a doubling time for another four *L. inermis* clones was
152 estimated to be 1.48-1.73 days at 20 °C (Fiałkowska et al. 2011). The size of eggs constitutes the
153 exceptionally large proportion of the female size, and consequently, the size of newly hatched
154 females is 71% of adult females on average (Miller 1931). *L. inermis* has been intensively
155 investigated regarding the phenomenon of the temperature-size rule. It follows this rule both in
156 the laboratory and in the field (Kiełbasa et al. 2014) and displays adaptive body size adjusted to
157 temperature-dependent oxygen levels (Walczyńska et al. 2015a) in a mechanism controlled at
158 two points within the lifecycle (Walczyńska et al. 2015b).

159 Details about the isolation and laboratory maintenance of the clones used in the study are
160 provided in Table 1. For each clone, the isolation was started from one individual fed with 25 μ l
161 of 3‰ molasses solution or 10 μ l of suspension of NOVO, a nutrition powder used for rotifer
162 mass culture (patented by Pajdak-Stós et al. 2017), 0.20 g NOVO in 50 ml of Żywiec spring
163 water. The proliferated clones were cultured in 24-well tissue culture plates in Żywiec brand
164 spring water (Poland), fed with NOVO and kept in darkness. Clones selected for high
165 performance at a lower temperature, specifically 8 °C or 15 °C, were transferred to the new wells
166 in the tissue culture plates every two weeks. Other clones were passaged every week. The clones
167 included in Table 1 are those showing the highest proliferation under such selection from among
168 all the clones tested. In 09/2016, all the clones, except for Warm2, were transferred to 25 °C
169 (darkness) in Żywiec medium-fed NOVO. We assumed that the best-performing clones
170 underwent some adaptive mechanisms to the given thermal conditions, and this condition-
171 specific selective force might have differentiated the clonal general life histories. Clone Warm2
172 was transferred from 20 °C to 25 °C in 06/2017. All clones were exclusively parthenogenetic,
173 which is typical of *L. inermis* from this type of habitat (Pajdak-Stós et al. 2014).

174 **Body and egg size.** For each clone, the subsamples were taken from the stock populations
175 maintained in common garden conditions at 25 °C and were fixed with Lugol solution.
176 Approximately 50 females and 30 eggs per temperature were photographed using the Nikon
177 Eclipse 80i microscope, Nikon DS-U1 camera and NIS Elements software. The length and width
178 were measured in ImageJ (NIH, USA), and their product was treated as an area measure.

179 **Fecundity.** In 06/2017, 60-mm Ø Petri dishes with a small number of females representing three
180 clones, Int2, Cold, and Warm2 (one dish per clone), were transferred to 30 °C and fed with 10 μ l
181 of the commercial bioproduct Biotrakt® (Zielone oczyszczalnie, Poland). The choice of
182 temperature was dictated by the results indicating that the optimal temperature for *Lecane inermis*

183 clone Lk6 was 31 °C (Walczyńska et al. 2016). After two days, which was necessary to obtain
184 the next-generation females from freshly laid eggs, 24 individual young females per clone were
185 transferred to separate wells in 24-well tissue culture plates (TPP, Switzerland) with 1 mL of
186 Żywiec medium and 10 µl of 10× diluted Biotrakt® per well. The number of eggs laid per female
187 was checked daily under a stereomicroscope. In one case, the gap between two checks was two
188 days. If eggs were observed, a female was transferred to a new well (with the same conditions as
189 previously described) to avoid the counting of eggs laid by the next-generation females. The
190 death of each female was noted. This procedure was continued until the last female died. After
191 completion of this stage, the same procedure was conducted for another three clones: Int1, Int3
192 and Warm1. The eggs laying was examined daily except for one case, where the gap between the
193 inspections was two days.

194 **Growth rate estimation.** In 07/2017, populations of all six clones were established in four
195 temperature treatments: 15, 20, 25 and 30 °C. The initial numbers were 10 females × three
196 replicates per clone. The cultures were kept in six-well tissue culture plates (TPP, Switzerland),
197 with Żywiec as the medium supplemented with 20 µL of Biotrakt®. The number of females was
198 counted twice, with a 1-2-day gap between the counts, to correct for growing processes being
199 physically dependent on temperature (a longer gap was applied at the two lower temperature
200 regimes). These numbers were then used to calculate the population growth rate r according to
201 the formula $r = (\ln(x_2) - \ln(x_1))/t$, where x_2 – number of individuals in count II, x_1 – number of
202 individuals in count I, and t – time in days.

203 **Size-to-temperature response examination.** In 08/2017, approximately 60 individuals per clone
204 per temperature, derived from the cultures maintained for growth rate estimation, were fixed with
205 Lugol solution for size measurements. The rotifers were photographed using a Nikon Eclipse 80i

206 microscope, Nikon DS-U1 camera and NIS Elements software. The lengths and widths of
207 individuals were measured using ImageJ (NIH, USA), and their product was used as a proxy for
208 area.

209 **Statistical analyses.** All the analyses were performed in Statistica 13 (StatSoft 2014). Parametric
210 tests were conducted when the assumptions were met. Under assumptions violation, the non-
211 parametric tests were conducted. In the case of size, simple regression was used to test for the
212 relationship between clone-specific female size and egg size. We tested for the differences in the
213 number of eggs laid per day of female life using the Kruskal-Wallis test. The differences in the
214 growth rate between clones were tested using one-way ANOVA for each temperature separately
215 (the lack of homogeneity of variance prevented joint analysis). The differences in body size
216 between clones (the plastic size responses) were tested using one-way ANOVA for each
217 temperature separately because of the violation of the assumption of the homogeneity of
218 variance. The same reason prevented parametric analysis at 30 °C. In this case, the differences
219 were tested with the Kruskal-Wallis test. To compare the temperature effect on growth rates
220 among the groups of thermal acclimation, one value describing a common slope of response
221 across temperature was estimated for each clone using the simple regression model (statistics
222 presented in Table 2), and then these values were compared among clones in a one-way ANOVA
223 for the groups of warm-, cold- and medium-temperature acclimation. The same procedure was
224 applied to compare the strength of TSR among the groups of thermal specialization, and the
225 statistics are presented in Table 2.

226

227 **Results**

228 **Body and egg size.** The female size in μm^2 and respective egg size for each clone are provided in
229 Table 3. There was no interclonal relationship between female size and egg size (slope = 0.15,

230 $R^2_{(adj)} = 0.025$, $p = 0.35$; Fig. 1A). The average egg size as a proportion of mother size reached
231 78% (from 73% for Int3 to 85% for Warm1).

232 **Fecundity and lifespan.** At the common temperature of 30 °C assumed optimal, clones differed
233 in the mean number of eggs laid by the females (Kruskal-Wallis test: $H_{(5, 125)}=15.3$, $p = 0.009$)
234 and in the female lifespan duration (Kruskal-Wallis test: $H_{(5, 125)}=13.9$, $p = 0.016$). The females
235 laid significantly more eggs with the lifetime duration (slope = 2.2, $R^2_{(adj)} = 0.76$, $p < 0.0001$),
236 with the Cold clone being the most fecund, followed by both warm clones and, finally, the least
237 fecund intermediate clones (Fig. 1B).

238 **Growth rate and temperature-size rule.** There were significant differences among clones in
239 growth rate at each temperature. According to the Tukey post hoc tests,

- 240 - 15 °C, Int2 clone had significantly faster growth than clone Warm2, while the other four
241 clones showed intermediate patterns;
- 242 - 20 °C, clones Int2 and Warm2 grew significantly faster than clone Int3;
- 243 - 25 °C, Cold clone performed significantly better than clones Int2, Int3 and Warm1;
- 244 - 30 °C, clone Warm1 grew faster than clones Warm2 and Int3, and clones Cold and Int1
245 performed better than clone Int3 (Fig. 2A).

246 All rotifers were smaller with higher temperature (slope = - 0.11, $R^2_{(adj)} = 0.34$, $p < 0.001$), and
247 significant differences among clones existed in all but the 30 °C treatment (Fig. 2B). Generally,
248 clone Int3 was the largest across the regimes, while the smallest were both warm clones. The
249 Cold clone presented an intermediate pattern together with the remaining two Int clones.
250 The analysis of the across-temperature ability to proliferate and to plastically respond (Table 2)
251 showed the opposite effect of temperature. The interclonal comparison of these two temperature-

252 dependent effects (with the strength of the size-to-temperature response presented as a positive
253 effect for the sake of clarity), with the clones pooled within the thermal acclimation groups (N =
254 3), showed a general lack of relationship (slope = -0.087, $R^2 = 0.198$, $p = 0.38$; Fig. 3). The
255 population growth rate was not different across specialization ($F_{(2,3)} = 1.39$, $p = 0.37$; Fig. 3, x-
256 axis), while a significant difference occurred in the case of the strength of TSR across groups
257 ($F_{(2,3)} = 27.53$, $p = 0.012$; Fig. 3, y-axis). Clones acclimated to intermediate temperature displayed
258 a significantly stronger (according to Tukey's HSD test) size-to-temperature response than warm-
259 acclimated clones, while Cold clone represented an intermediate pattern.

260

261 **Discussion**

262 In this study, we present the interclonal link between thermal preference, ability of plastic
263 response and fitness to elucidate the issue of ecological trade-offs. First, in hypothesis H1, we
264 tested whether thermal acclimation could be referred to as thermal preference in the six
265 investigated clones. According to our predictions, the cold-acclimated clone was larger than the
266 two warm-acclimated clones (Fig. 1A). The three clones acclimated to intermediate temperature
267 represented a wide range of sizes, although they were consistently larger across temperatures than
268 warm-acclimated clones (Fig. 1A), also in accordance with our predictions. We expected the
269 Cold clone to be the largest, which was not the case; however, based on the size measurements
270 from the stock populations, only one Int clone appeared larger than the Cold clone (Fig. 1A).
271 Therefore, we generally validated our assumption that it is justified to refer to thermal preference
272 instead of thermal acclimation in our further interpretation of the results. Additionally, this result
273 confirms the adaptive nature of body size linked to temperature at the interclonal level along with
274 the previous interspecific patterns found for three species representing the *Brachionus plicatilis*

275 cryptic species complex (Walczyńska and Serra 2014) and five species of free-living nematodes
276 (Majdi et al. 2019).

277 Regarding hypothesis H2 on the relationship between thermal preference and fitness across
278 temperature, warm clones were the poorest performers at cold temperatures and were
279 considerably better at higher temperatures, especially Warm1 (Warm2 appeared to have a thermal
280 optimum closer to 25 °C than to 30 °C; Fig. 2A). The cold-acclimated clone performed notably
281 well at high temperatures but was an intermediate performer at the lowest temperature. Int-clones
282 showed different patterns, with Int1 and Int2 performing relatively well at all temperatures (a
283 clear generalist strategy) and Int3 performing poorly in all thermal regimes (Fig. 2A). Our results
284 suggest that specialization is restricted to warm-acclimated organisms rather than to mid-
285 temperature-acclimated organisms, which present the strategy of generalists, thus partly
286 confirming our predictions in H2. The case of the Cold clone is more complicated. At the lowest
287 temperature, it had higher fitness than warm-acclimated clones but similar or lower fitness than
288 Int clones. However, it is important to note that the lowest experimental temperature was not the
289 one to which a Cold clone was acclimated, and using a temperature below 15 °C might result in
290 the Cold clone displaying higher fitness than other clones, the prediction supported by the pilot
291 study presented in the supplementary materials (Fig. S1). Such a thermal regime was not planned
292 in the study because it was previously reported that the minimum temperature for the
293 proliferation of a clone referred to here as Warm1 was 11 °C (Walczyńska et al. 2016).

294 Therefore, we set the lowest thermal regime to 15 °C to compare the positive proliferation of all
295 six clones. The result we obtained for a Cold clone confirms the classical models in which
296 organisms acclimated to the use of a wider range of resources perform better (originally, they
297 outcompete) those of a narrower range (Tilman 1982). Our Cold clone was acclimated to the
298 usage of resources at the lowest thermal regimes (down to 8 °C), unlike all the others. This result

299 is more in keeping with the findings described by Zamorano et al. (2017), with cold-preferring
300 organisms performing notably well in the whole range studied, than with the findings reported by
301 Majdi et al. (2019), with the largest species performing well only in the coldest regime. Perhaps
302 there are other factors that cause the discrepancies among these studies; one of the most possible
303 factors is body size. Our results on clone-specific body size show that it is better not to be the
304 largest but rather to have an intermediate size, unless the temperature is high. In this way, we
305 solved the conflict in the haiku in Kingsolver and Huey (2008): *Bigger is better. And hotter*
306 *makes you smaller. Hotter is better.* The solution is that hotter is better, but only for smaller, a
307 result previously achieved for the *L. inermis* rotifer (Walczyńska et al. 2015a). This result was
308 obtained in the context of oxygen as a driving factor in the TSR, as theoretically assumed
309 (Atkinson et al. 2006; Verberk et al. 2011), and provides one of the limited confirmations of the
310 adaptive role of small size under a combination of high temperature and low oxygen availability.
311 In this study, six clones of *Lecane inermis* rotifer displayed a consistent decrease in size with
312 increasing temperature regardless of their thermal acclimation and the resulting thermal
313 preferences. The relationship between the strength of this response and thermal specialization
314 (hypothesis H3) showed that clones acclimated to medium temperature displayed a stronger
315 decrease in size with increasing temperature than cold- and warm-specialized clones (Table 2,
316 Fig. 2B). This pattern was not a compensation of size plasticity for the thermal sensitivity of the
317 population growth rate because their relationship indicating a possible trade-off was not
318 significant (Fig. 3). However, this result may be attributable to a small sample size, and the
319 possibility of such compensation cannot be fully excluded. Both Cold and Warm clones
320 displayed a weaker size-to-temperature response than Int clones, showing that the strength of this
321 plastic reaction is not just a linear effect of acclimation temperature.

322 The thermal regimes were chosen to reflect the optimal thermal range previously invoked to
323 designate the frames for TSR performance (Atkinson et al. 2003; Walczyńska et al. 2016). Thus,
324 the responses we examined were not the result of exposure to stressful conditions on any end of
325 thermal range, and they therefore did not deviate from the TSR in a classical sense. In addition,
326 we link this pattern with thermal preferences and the level of thermal specialization. We show for
327 the first time that organisms acclimated to intermediate temperature performed relatively well
328 across temperatures, thus confirming the theoretical prediction that intermediate phenotypes as
329 generalists (DeWitt and Langerhans 2004) display stronger TSR responses than specialists. This
330 result presents the first empirical confirmation of the previous speculations made for three
331 *Brachionus plicatilis* sister species (Walczyńska and Serra 2014).

332 The set of hypotheses stems from the theoretical predictions on generalist-specialist trade-offs in
333 the context of adaptation to thermal conditions. Accordingly, the most beneficial temperature is
334 expected to be the one to which an organism was acclimated (beneficial acclimation hypothesis,
335 BAH; Leroi et al. 1994). Alternatively, the adaptation may refer only to low temperature
336 acclimation (colder is better, CIB), hot acclimation (hotter is better, HIB), optimal (intermediate)
337 temperature (optimal developmental temperature hypothesis), and other variants (reviewed in
338 Deere and Chown 2006). However, none of the variants was found to be universal. Our results
339 generally confirm the beneficial acclimation hypothesis (BAH) – clones exposed for a long time
340 to specific thermal conditions display a preference (= perform better) for these conditions – but
341 this effect is not consistent within a thermal continuum. The second-to-largest Cold clone was the
342 best performer across the regimes, confirming the results found previously for parasitoids
343 (Zamorano et al. 2017) and thereby confirming the colder-is-better hypothesis (CIB). On the
344 other hand, Warm clones, the smallest, confirm the previous positive results for hotter-is-better
345 (HIB) (Kingsolver 2009; Knies et al. 2009). The difference in these two clones is that Warm1

346 was previously reported to have a very high optimal temperature despite its origin from a
347 temperate region, while Warm2 was assumed to be Warm-acclimated because of its origin from
348 the Mediterranean region (Table 1). Interestingly, in light of our results, the Warm1 clone was
349 more specialized at high temperatures than Warm2. Two mid-sized medium temperature clones
350 performed relatively well and evenly in the thermal range studied, reflecting a clear generalist
351 pattern, and a similar result was obtained for mid-sized nematode species (Majdi et al. 2019). Our
352 largest clone, also acclimated to intermediate temperature, exhibited the poorest performance of
353 all (though best at the lowest temperature as relative to other clones). Regarding nematodes, the
354 largest species showed notably poor growth in all other regimes compared with the other four
355 species, although it had the highest fitness in the coldest temperature (Majdi et al. 2019). These
356 results are in contrast to previous reports (Geister and Fischer 2007; Kingsolver and Huey 2008)
357 and show that larger sizes are not always better. Our novel contribution to the discussion of the
358 superiority of the BAH, CIB or HIB hypotheses is that the answer is context-dependent, and that
359 context is the level of thermal specialization of the investigated organisms.

360 The additional information on the reproductive traits shed light on the interclonal differences in
361 general life strategies. In contrast to the optimal theory models (Fox and Czesak 2000; Honěk
362 1993; Parker and Begon 1986), we found no interclonal trade-off between female size and egg
363 size (Fig. 1A) or between female lifespan and fecundity (Fig. 1B), though such trade-offs cannot
364 be excluded at the level of individuals. The most apparent trade-off we found was allocation in
365 reproduction *vs.* ability to proliferate across temperature between specialists and generalists. The
366 cold-acclimated clone was the most fecund, and the two smallest, warm-acclimated clones
367 displayed an intermediate pattern, while intermediate temperature-acclimated clones displaying
368 the generalist strategy (evenly good performance at all temperatures) were the shortest-living and

369 thus the least fecund at the temperature assumed to be optimal (Fig. 1B). Therefore, the
370 specialization showed some signs of the *jack of all trades, master of none* (Huey and Hertz,
371 1984) pattern; clones specialized to warm performed either extremely poorly at the lowest
372 temperature (clone Warm2) or extremely well at the highest temperature (clone Warm1), while
373 two of the medium clones, with the exception of the largest one, performed relatively well across
374 all of the regimes. The interpretation of cold preference is not straightforward because we had
375 only one clone previously acclimated to low temperature. However, our results on the population
376 growth rate (including the thermal extension from a pilot study provided in the supplement) as
377 well as the data on fecundity together suggest that our cold-preferring clone may also be
378 perceived as a specialist.

379 The results showed that 30 °C was optimal (= gave the highest mean performance) for two
380 clones, Warm1 and Int2. However, in the case of the remaining three clones, the difference in
381 performance between 25 °C and 30 °C was relatively small, and in each case, the value achieved
382 at 30 °C was much higher than that at 20 °C (Fig. 2A). Therefore, the discrepancy between 30 °C
383 and the real optimal temperature for all six clones should not considerably affect the results on
384 fecundity, which we assumed to measure at the optimum.

385 Our results provide a new scientific perspective on the issue of the performance of generalists vs.
386 specialists: specialists allocate more in reproduction and are therefore more fecund than
387 generalists at optimal temperatures, while generalists display stronger plastic size response to
388 temperature (the temperature-size rule). This result shows a possible empirical solution to the
389 distinction between specialists and generalists first introduced by Levins (1968): specialists
390 allocate more in reproduction at optimal temperatures, while generalists invest in plastic
391 responses across environments.

392 **References**

- 393 Acerenza L (2016) Constraints, Trade-offs and the Currency of Fitness. *J Mol Evol* 82:117-127
- 394 Atkinson D (1994) Temperature and organism size – a biological law for ectotherms. *Adv Ecol*
395 *Res* 25:1-58
- 396 Atkinson D, Ciotti BJ, Montagnes DJ (2003) Protists decrease in size linearly with temperature:
397 ca. 2.5% degrees C(-1). *Proc Royal Soc B* 270:2605-11
- 398 Atkinson D, Morley SA, Hughes RN (2006) From cells to colonies: at what levels of body
399 organization does the 'temperature-size rule' apply? *Evol Dev* 8:202-214
- 400 Berger D, Walters RJ, Blanckenhorn WU (2014) Experimental evolution for generalists and
401 specialists reveals multivariate genetic constraints on thermal reaction norms. *J Evol Biol*
402 27:1975-1989
- 403 Berrigan D, Charnov EL (1994) Reaction norms for age and size at maturity in response to
404 temperature – a puzzle for life historians. *Oikos* 70:474-478
- 405 Bielańska-Grajner I, Ejsmont-Karabin J, Radwan S (2015) Rotifers. *Rotifera Monogononta*. Lodz
406 University Press and Jagiellonian University Press, Lodz-Krakow.
- 407 Bruijning M, Berge ACM, Jongejans E (2018) Population-level responses to temperature, density
408 and clonal differences in *Daphnia magna* as revealed by integral projection modelling.
409 *Funct Ecol* 32:2407-2422
- 410 Cody ML (1966) A general theory of clutch size. *Evolution* 20:174-184
- 411 Deere JA, Chown SL (2006) Testing the beneficial acclimation hypothesis and its alternatives for
412 locomotor performance. *Am Nat* 168:630-644

- 413 DeWitt TJ (2016) Expanding the phenotypic plasticity paradigm to broader views of trait space
414 and ecological function. *Curr Zool* 62:463-473
- 415 DeWitt TJ and Langerhans RB (2004) Integrated solutions to environmental heterogeneity:
416 theory of multimoment reaction norms. In *Phenotypic plasticity: functional and*
417 *conceptual approaches.*, eds. TJ DeWitt and SM Scheiner, pp. 98-111. Oxford: Oxford
418 University Press.
- 419 Fiałkowska E, Kocerba W, Pajdak-Stós A, Klimek B, Fyda J (2011) Clonal variation in
420 reproductive response to temperature by a potential bulking control agent *Lecane inermis*
421 (Rotifera). *Water Sci Technol* 64:403-408
- 422 Fields PA (2001) Review: Protein function at thermal extremes: balancing stability and
423 flexibility. *Comp Biochem Physiol A* 129:417-431
- 424 Foray V, Gibert P, Desouhant E (2011) Differential thermal performance curves in response to
425 different habitats in the parasitoid *Venturia canescens*. *Naturwissenschaften* 98:683-691
- 426 Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. *Annu Rev*
427 *Entomol* 45:341-369
- 428 Geister TL, Fischer K (2007) Testing the beneficial acclimation hypothesis: temperature effects
429 on mating success in a butterfly. *Behav Ecol* 18:658-664
- 430 Griffith TM, Sultan SE (2006) Plastic and constant developmental traits contribute to adaptive
431 differences in co-occurring *Polygonum* species. *Oikos* 114:5-14
- 432 Hildrew AG, Raffaelli D, Edmonds-Brown R (2007) Body size: the structure and function of
433 aquatic ecosystems. Cambridge University Press, Cambridge.
- 434 Hoefnagel KN, de Vries EHJ, Jongejans E, Verberk WCEP (2018) The temperature-size rule in
435 *Daphnia magna* across different genetic lines and ontogenetic stages: multiple patterns
436 and mechanisms. *Ecology and Evolution* 8: 3828–3841

- 437 Honěk A (1993) Intraspecific variation in body size and fecundity in insects - a general
438 relationship. *Oikos* 66(3):483-492 doi:10.2307/3544943.
- 439 Huey RB, Hertz PE (1984) Is a jack-of-all-temperatures a master of none? *Evolution* 38:441-444
- 440 Johnston IA, Bennett AF (2008) *Animals and temperature. Phenotypic and Evolutionary*
441 *Adaptation*. Cambridge University Press.
- 442 Kielbasa A, Walczyńska A, Fiałkowska E, Pajdak-Stós A, Kozłowski J (2014) Seasonal changes
443 in the body size of two rotifer species living in activated sludge follow the temperature-
444 size rule. *Ecol Evol* 4:4678-4689
- 445 Kingsolver JG (2009) The Well-Tempered Biologist. *Am Nat* 174:755-768
- 446 Kingsolver JG, Huey RB (2008) Size, temperature, and fitness: three rules. *Evol Ecol Res*
447 10:251-268
- 448 Klimowicz H (1989) Znaczenie mikrofauny przy oczyszczaniu ścieków osadem czynnym (in
449 Polish). Komitet Wydawniczy Instytutu Ochrony Środowiska, Warsaw.
- 450 Knies JL, Kingsolver JG, Burch CL (2009) Hotter Is Better and Broader: Thermal Sensitivity of
451 Fitness in a Population of Bacteriophages. *Am Nat* 173:419-430
- 452 Kozłowski J (1992) Optimal allocation of resources to growth and reproduction - implications for
453 age and size at maturity. *TREE* 7:15-19
- 454 Kozłowski J (2006) Why life histories are diverse. *Polish J Ecol* 54:585-605
- 455 Lailvaux SP, Husak JF (2017) Predicting Life-History Trade-Offs with Whole-Organism
456 Performance. *Integr Comp Biol* 57:325-332
- 457 Leroi AM, Bennett AF, Lenski RE (1994) Temperature-acclimation and competitive fitness - an
458 experimental test of the beneficial acclimation assumption. *PNAS* 91:1917-1921
- 459 Levins R (1968) *Evolution in Changing Environments. Some Theoretical Explorations*. Princeton
460 University Press, Princeton.

- 461 Majdi N, Traunspurger W, Fueser H, Gansfort B et al (2019) Effects of a broad range of
462 experimental temperatures on the population growth and body-size of five species of free-
463 living nematodes. *J Therm Biol* 80:21-36
- 464 Miller HM (1931) Alternation of generations in the rotifer *Lecane inermis* Bryce. I. Life
465 histories of the sexual and non-sexual generations. *Biol Bull* 60:345-381
- 466 Pajdak-Stós A, Fiałkowska E, Fyda J, Kocerba-Soroka W et al (2017) A method of mass culture
467 of *Lecane* rotifers. International patent number EP 14731401.7
- 468 Pajdak-Stós A, Fiałkowska E, Kocerba-Soroka W, Sobczyk M et al (2014) Why is sex so rare in
469 *Lecane inermis* (Rotifera: Monogononta) in wastewater treatment plants? *Invertebr Biol*
470 133:128-135
- 471 Parker GA, Begon M (1986) Optimal egg size and clutch size: effects of environment and
472 maternal phenotype. *Am Nat* 128:573-592
- 473 Roff DA. (1992) *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New
474 York
- 475 Roff DA, Fairbairn DJ (2007) The evolution of trade-offs: where are we? *J Evol Biol* 20:433-447
- 476 Sheth SN, Angert AL (2014) The evolution of environmental tolerance and range size: a
477 comparison of geographically restricted and widespread *Mimulus*. *Evolution* 68:2917-
478 2931
- 479 StatSoft (2014) *STATISTICA* (data analysis software system). 12 edn.
- 480 Stearns SC (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
- 481 Tilman D (1982) *Resource Competition and Community Structure*. Princeton University Press,
482 Princeton.

- 483 Verberk WCEP, van der Velde G, Esselink H (2010) Explaining abundance-occupancy
484 relationships in specialists and generalists: a case study on aquatic macroinvertebrates in
485 standing waters. *J Animal Ecol* 79:589-601
- 486 Verberk WCEP, Bilton DT, Calosi P, Spicer JI (2011) Oxygen supply in aquatic ectotherms:
487 Partial pressure and solubility together explain biodiversity and size patterns. *Ecology*
488 92:1565-1572
- 489 Walczyńska A, Kiełbasa A, Sobczyk M (2016) 'Optimal thermal range' in ectotherms: defining
490 criteria for tests of the temperature-size rule. *J Therm Biol* 60:41-48
- 491 Walczyńska A, Labecka AM, Sobczyk M, Czarnoleski M et al (2015a) The Temperature-Size
492 Rule in *Lecane inermis* (Rotifera) is adaptive and driven by nuclei size adjustment to
493 temperature. *J Therm Biol* 54:78-85
- 494 Walczyńska A, Serra M (2014) Inter- and intraspecific relationships between performance and
495 temperature in a cryptic species complex of the rotifer *Brachionus plicatilis*.
496 *Hydrobiologia* 734:17-26
- 497 Walczyńska A, Sobczyk M, Czarnoleski M, Kozłowski J (2015b) The temperature-size rule in a
498 rotifer is determined by the mother and at the egg stage. *Evol Ecol* 29:525-536
- 499 Zamorano J, Bozinovic F, Veloso C (2017) Colder is better: The differential effects of thermal
500 acclimation on life history parameters in a parasitoid fly. *J Therm Biol* 68:1-4

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503 **Table 1.** Description of the origin and the thermal conditions of the maintenance (= acclimation)
504 preceding this study for the experimental clones of *Lecane inermis* rotifer. WWTP – wastewater
505 treatment plant

code	description	thermal preferences
Int1	isolated in WWTP1 in Poland in 2014, maintained at 15 °C	intermediate
Int2	isolated in WWTP1 in Poland in 2014, maintained at 15 °C	intermediate
Int3	isolated in WWTP1 in Poland in 2014, maintained at 15 °C	intermediate
Warm1	isolated in WWTP2 in Poland in 2010, maintained at 20 °C	warm ^a
Warm2	isolated in WWTP near Barcelona, Spain in 2011, maintained at 20 °C	warm
Cold	isolated in WWTP3 in Poland in 2015, maintained at 8-10 °C for 10 months, later transferred to 15 °C	cold

506 ^aoptimal temperature estimated at 31 °C by Walczyńska et al. (2016)

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514 **Table 2.** Statistics for the estimation of the slopes for population growth rate and size across
 515 temperature.

trait	Clone	slope	R²_(adj)	t (df)	p
population growth rate	Cold	0.083	0.65	4.63 (10)	0.00093
	Int1	0.068	0.82	7.07 (10)	0.00003
	Int2	0.041	0.69	5.03 (10)	0.00051
	Int3	0.028	0.29	2.27 (9)	0.04964
	Warm1	0.084	0.79	6.42 (10)	0.00008
	Warm2	0.053	0.71	5.22 (10)	0.00039
TSR	Cold	-0.021	0.33	-8.33 (140)	< 0.000001
	Int1	-0.022	0.43	-9.09 (108)	< 0.000001
	Int2	-0.025	0.52	-11.39 (117)	< 0.000001
	Int3	-0.023	0.29	-6.52 (101)	< 0.000001
	Warm1	-0.015	0.27	-7.23 (136)	< 0.000001
	Warm2	-0.014	0.22	-5.75 (111)	< 0.000001

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521 **Table 3.** The mean body and egg size for studied clones for individuals sampled from the
522 common garden stock at 25 °C. Ordered from the smallest to the largest clone.

clone	Female size (μm^2)	Egg size (μm^2)
	mean \pm SE; N	mean \pm SE; N
Warm1	2517 \pm 32; 55	2147 \pm 25; 30
Warm2	2665 \pm 46; 55	2098 \pm 25; 29
Int2	2799 \pm 40; 55	2201 \pm 28; 30
Int1	2812 \pm 42; 54	2078 \pm 34; 29
Cold	2848 \pm 56; 54	2182 \pm 24; 30
Int3	3019 \pm 54; 53	2210 \pm 33; 24

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533 **Figure legends**

534 **Fig. 1.** Relationships among life history traits for six clones of *Lecane inermis* rotifer: female
535 body size and egg size measured from the stock populations at 25 °C (A), female lifespan and
536 total number of eggs laid per female estimated at the temperature of 30 °C assumed optimal (B).
537 Thermal adaptation distinguished by color. Means \pm SE

538 **Fig. 2.** Interclonal response to temperature in six clones of *Lecane inermis* rotifer: population
539 growth rate r (A) and temperature-size rule response (B). Means \pm SE.

540 **Fig. 3.** Relationship between the slopes of the following: increase in population growth rate r (x)
541 and of body size decrease (the absolute values of the negative slopes; y) across temperature. Each
542 point represents a clone with its thermal adaptation distinguished by color. slope \pm SE

Fig. 1.

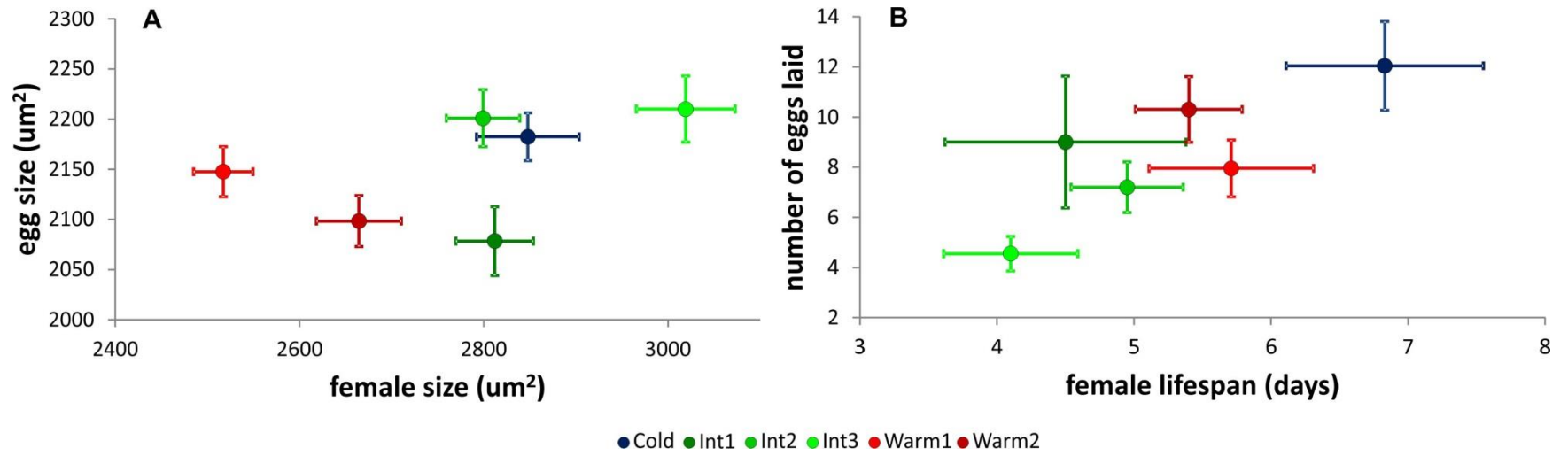


Fig. 2.

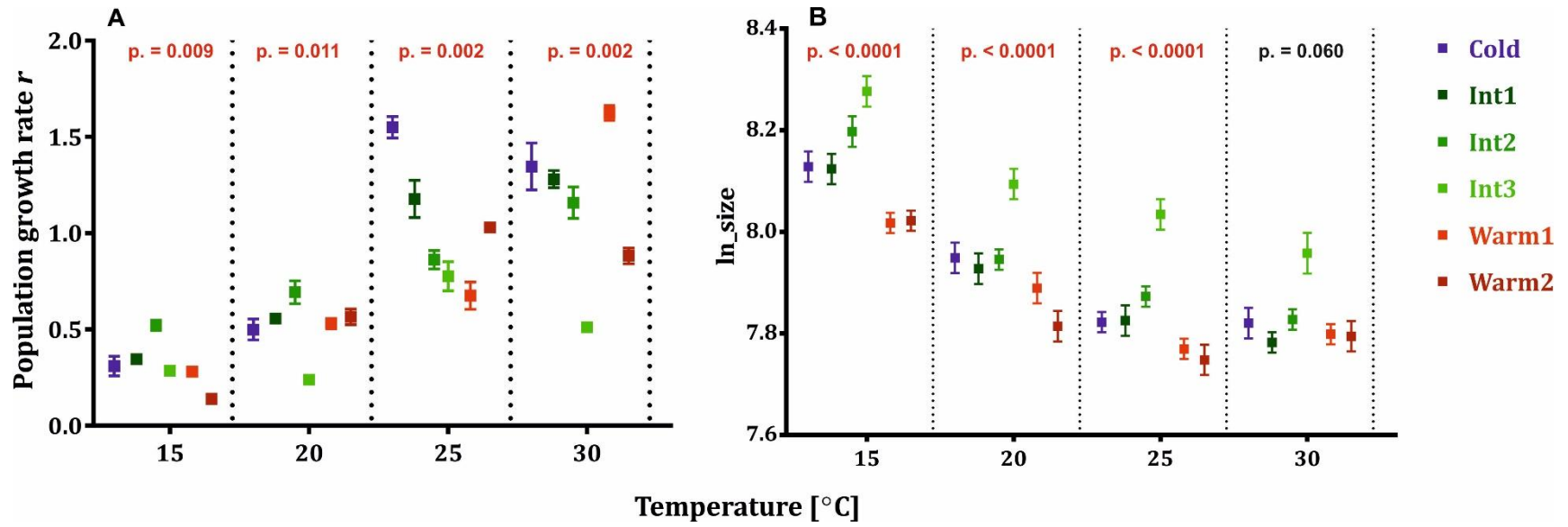


Fig. 3.

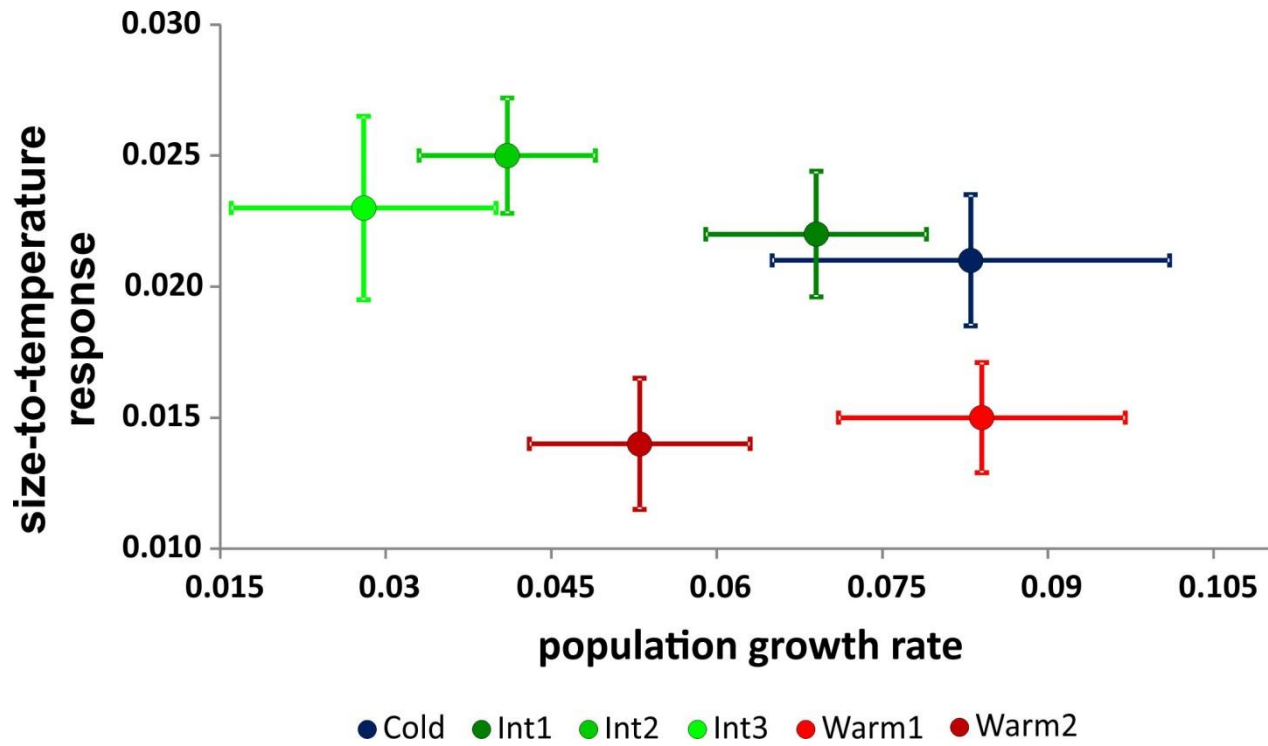


Figure S1. Pilot results (J. Starzycka, unpublished) for the three clones out of six investigated in this report. The population growth rate was estimated at 8, 15 and 20 °C, in the 10 three-day periods, for eight replicates per clone. The data show the mean value from nine estimates (excluding the first, 0-3 days estimate), across the replicates. Mean \pm SD

